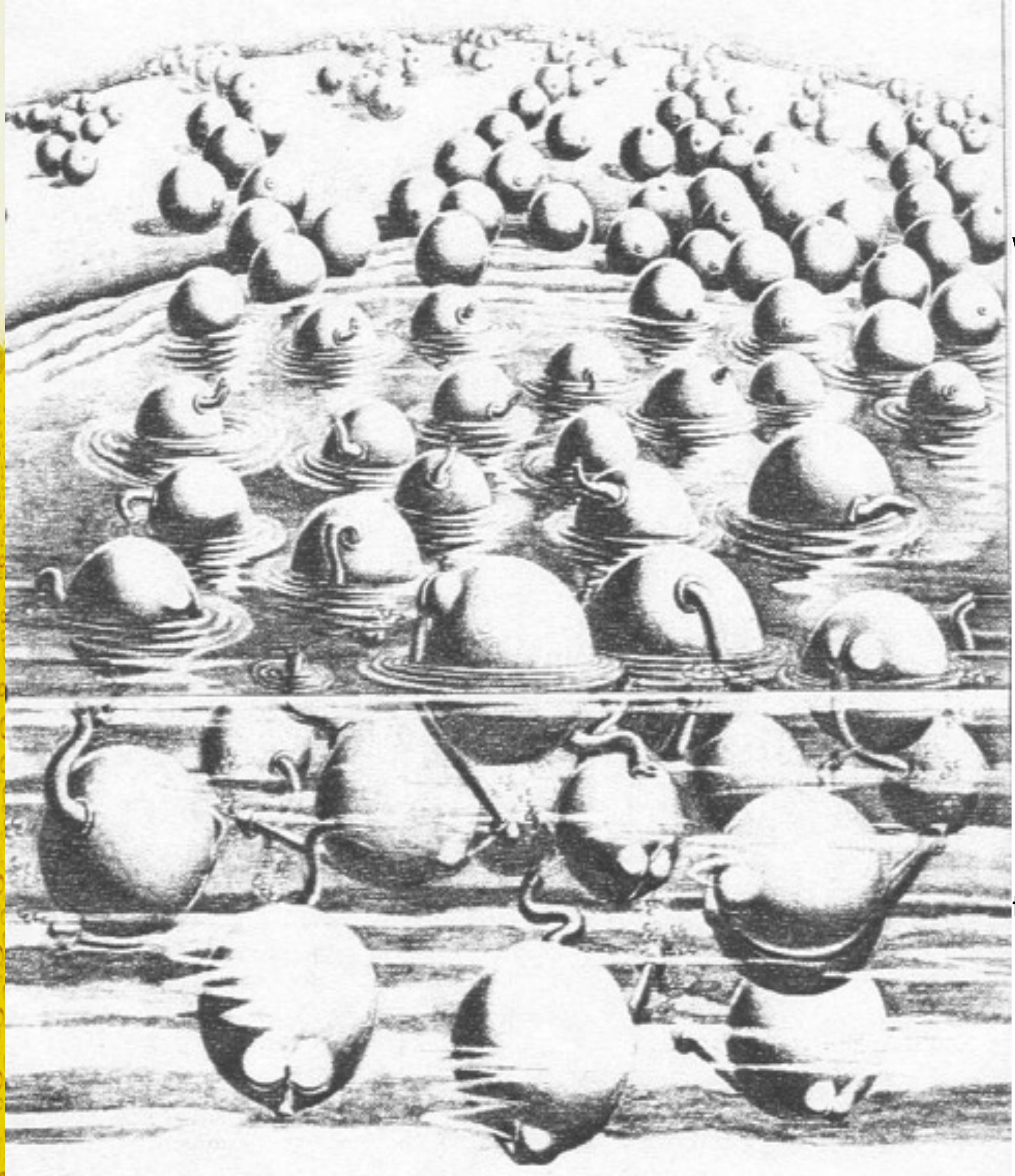
Yeast Metabolism
&
Fermentation By-Products
Influence on fermentation and product quality


GHOST WHISPERER



"Brewer's yeast dissolved in water disintegrates in countless, tiniest beads.
Upon adding them to sugared water the magic begins and small animals begin to form.
With their tiny suction spouts they eagerly suck up sugar from this solution whereupon immediate and unmistakable digestion sets in, characterised by spontaneous release of excrements from their bowels.
They excrete ethyl alcohol from their intestines and carbonic acid from their urinary tract.
Come, take a closer look at them. Do you see the incessant stream of a specifically lighter liquid rising from their anus and the gushes of carbonic acid being spurted out from their enormous genitals in short intervals?"

Liebig, Justus v. (1803-1875)

Uni-düsseldorf.de

Biochemical Changes during Fermentation

1. Fermentation of carbohydrates
2. Nitrogen in wort

    Assimilation/ Dissimilation

3. Formation of metabolic compounds

    Acids
    - CO_2
    - Organic acids

    Alcohols
    - Ethanol
    - Secondary and tertiary alcohols
    - Higher aliphatic alcohols (HAA)
    - Aromatic alcohols

    Esters
    Aldehydes and Ketones
    Vicinal Diketones (VDK)
    Sulphur-containing compounds

Changes during Fermentation

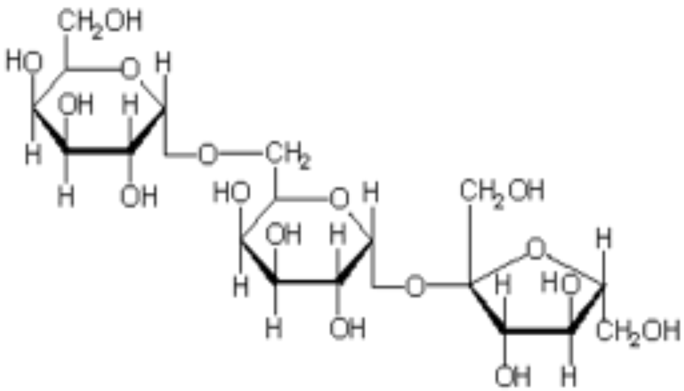
1. Decrease of pH
2. Decrease of bitter substances
3. Precipitation of nitrogen compounds
4. Decrease in viscosity
5. Decrease in redox potential
6. Changes in colour

Influences on Fermentation

- Aeration of the wort
- Wort concentration
- Fermentation temperature
- Amount of yeast (pitching rate)
- Yeast type
- CO_2-pressure
- Fermentation vessel
- Stirring (agitation)
- Additives (enzymes, minerals...)

Bottom and Top Fermenting Yeast Strains

- **Bottom fermenting yeast**
  - Fermentation at lower temperatures (4-10°C)
  - Forms aggregates at the end of fermentation (flocculation)
  - Ability of degrading raffinose completely
- **Top fermenting yeast**
  - Fermentation at higher temperatures (15-20°C)
  - No ability to ferment raffinose (galactose, glucose, fructose)
  - Produces less aroma components, especially esters (in comparison to bottom fermenting yeasts at same low temperatures)



Fermentation

Glucose
$(C_6H_{12}O_6)$

	$C_6H_{12}O_6$	→	$2\ C_2H_5OH$	+	$2CO_2$	+	235kJ/mol
100g		→	51.1g	+	48.9g		

(cell free fermentation)

Crabtree Effect

(Glucose effect, Pasteur counter-effect)

Respiration is partially inhibited by the starting glycolysis with a sugar surplus in the substrate → > 0,1 % (1 g/l)

" High rate of glycolysis reduces the respiration and induces fermentation"

- catabolite repression of respiratory chain enzymes
- slow response – gene level

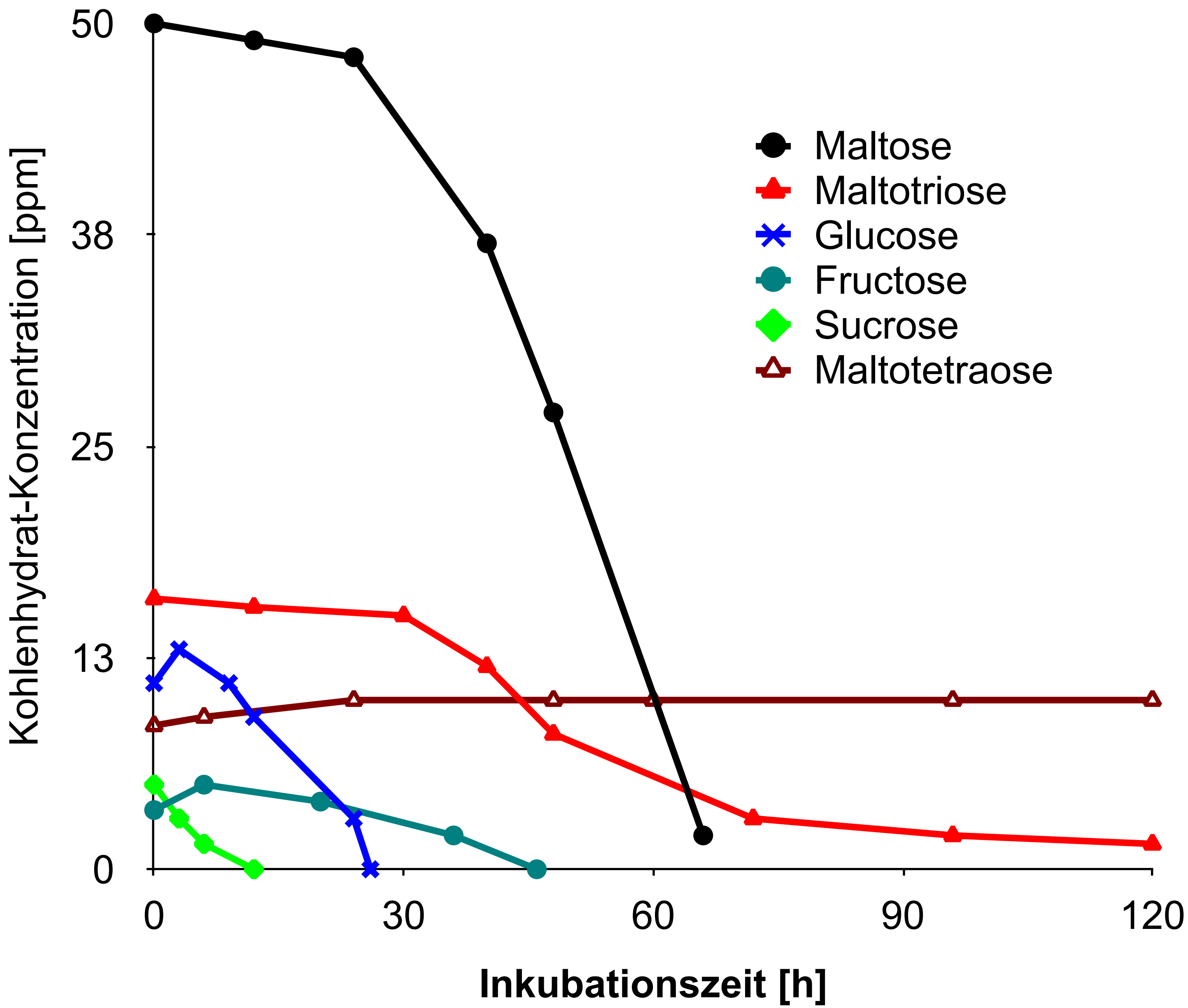
Overflow metabolism (short-term crabtree effect)

" High rate of glycolysis results in by-product formation from pyruvate"

- different mechanisms
- different functions
- fast response – enzyme regulation level

Source: Teknisk mikrobiologi 2003

Sugar Utilization



Amino Acid Synthesis by Yeast

- Total amino acid content in wort depends on the malting process and on variety and n-content of the used barley

- Amino acids are essential for the nutrition of the yeast

➔ good fermentation

➔ Good yeast growth

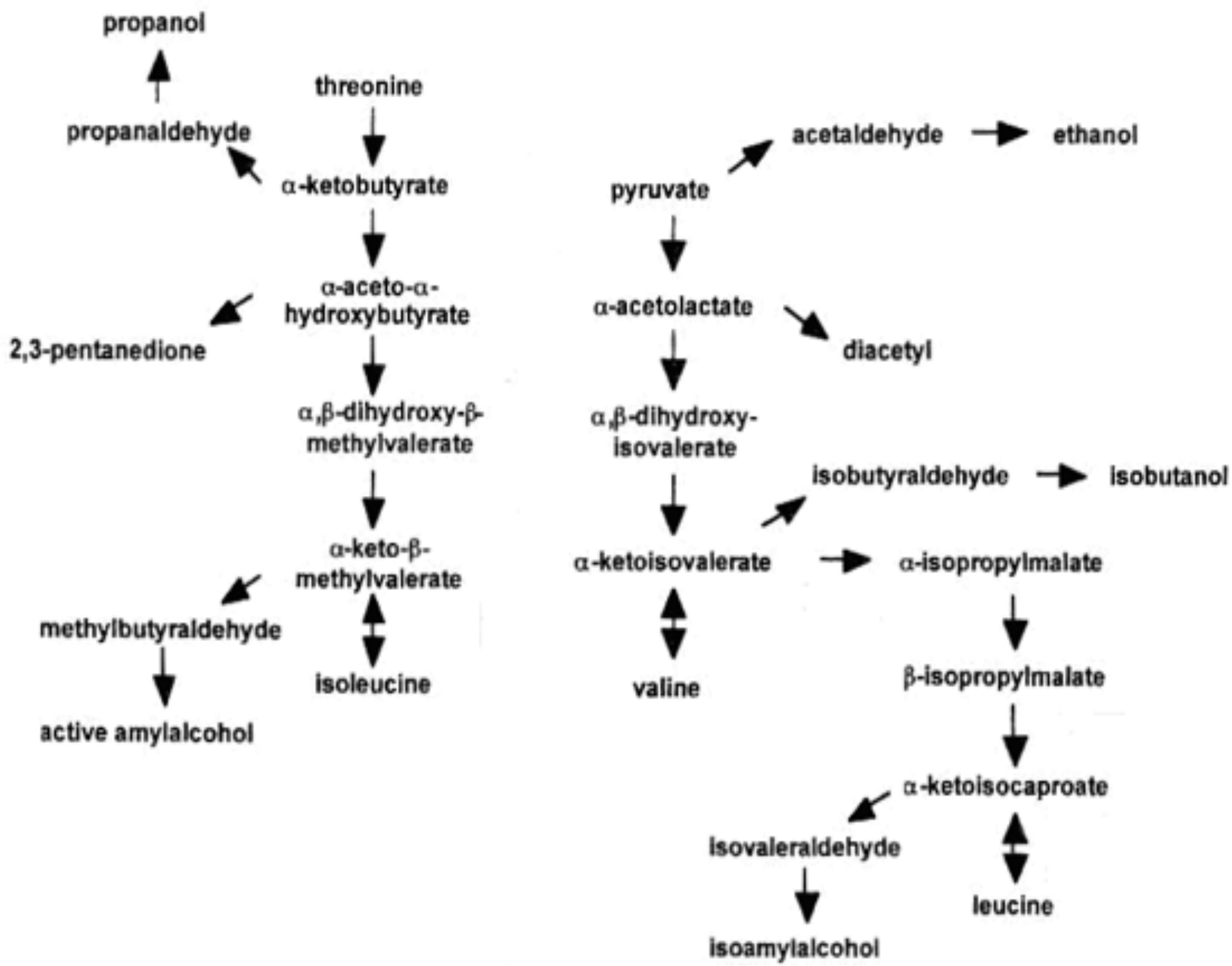
➔ good flavour and taste

- High quality fermentation ➔ certain content of amino acids in the wort is necessary

➔ Neither a surplus nor a shortage of amino acids is good ➔ increase of fermentation by products

Amino Acids and their Influence on Beer Aroma

Precursor amino acid	Strecker-aldehyd	Aroma
Leucine	3-Methylbutanal	malty
Isoleucine	2-Methylbutanal	malty
Methionine	Methional	Cooked potatoes
Phenylalanine	Phenylacetaldehyd	Honey like, floral



EBC Monograph 28, S. 60 – 73, 1999

Higher Alcohols [ppm]

	Normal values	Threshold
1. Aliphatic alcohols		
n-Propanol	5 – 33	600
Isobutyl alcohol	3 – 29	160
Isoamyl alcohol	27 – 95	110
2. Aromatic alcohols		
β-Phenyl ethanol	6 – 44	100

Technological Parameters Influencing the Values of HAA

- Yeast strain
- Pitching rate
- Wort aeration
- Fermentation temperature
- FAN
- Pressure
- Biochemical factors

Yeast Strain – HAA Formation

- Differences in formation of HAA are depending on physiological properties
- Variations in enzymatic behaviour
- Top fermenting yeasts normally produce more HAA than bottom fermenting yeast strains ➔ fermentation conditions!!!

Wort Aeration – HAA Formation

- Surplus aeration of wort usuallyleads to an increased formation of HA
- Smallest concentration in normal aerated worts:
  - Assumed amounts of oxygen:
    - Superaerated wort (17 mg O_2/l)
    - Normal aerated wort (8 – 9 mg O_2/l)
    - Subaerated wort (up to 1 mg O_2/l)
- Formation of amylalcohols is supported by a higher oxygen saturation of the wort

Effect of Increased Aeration Rates of Wort

- Increased yeast multiplication
- Higher intensity of fermentation
- More acetaldehyde formation
- More higher alcohol formation
- Increased ester formation
- Higher α-acetolactate formation resulting in more diacetyl
- Decreased free short chain fatty acids

Wort Aeration and Higher Alcohols

Aeration	8 ppm	12 ppm	0 ppm	Repeated aeration
n-Propanol	8.2	10.5	5.9	22.0
Isopropanol	12.0	10.9	10.0	15.2
2-Methylbutanol-(1)	15.5	16.7	7.9	17.6
3-Methylbutanol-(1)	54.9	57.1	33.7	88.2
Higher alcohols	90.6	95.2	57.5	143.0

Fermentation Temperature – HAA Formation

- Increase of the fermentation temperature ➔ increase of concentration of the HA

- Impact on the single alcohols differs

- Correlation between temperature and the supply of nutrients:
  - Demand for nitrogen is increased by higher growth promoting temperatures

  ➔ Lack of nitrogen ➔ higher production of HA by the anabolic pathway

- Influence of the temperature is important for the uptake of the amino acids
  - higher fermentation temperatures (12,5 °c) result in increase of HAA
- Increased yield of amylalcohols by increasing temperatures can be reduced by fermentation under pressure (1,8 bar)

- Not possible for n-Propanol and Isobutanol

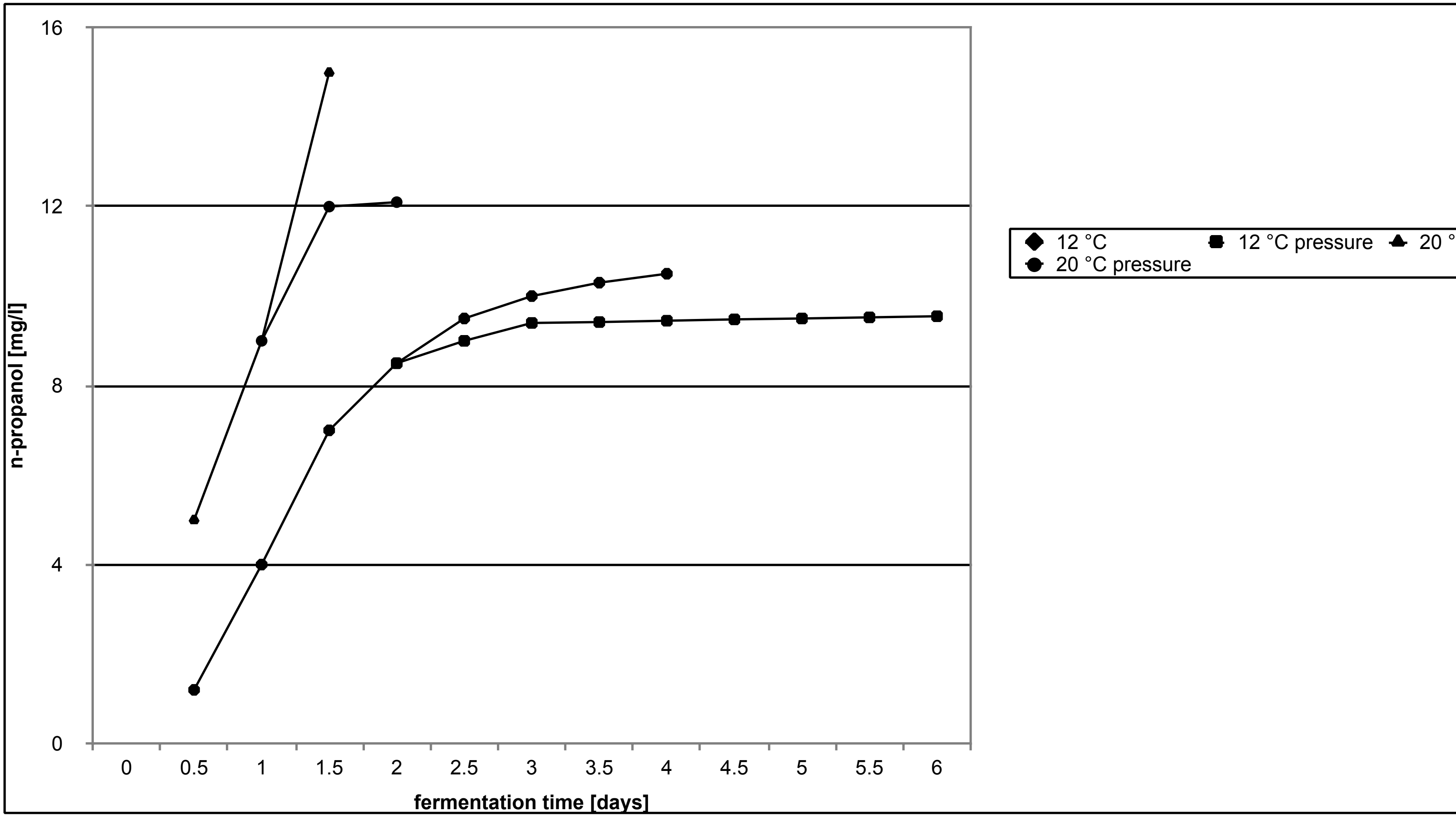
- Limiting (indicating) factor of fermentation is the Diacetyl

Pressure – HAA Formation

- High influence of pressure on formation of fermentation by-products
- Measures which lead to an excessive carbohydrate- and amino acid metabolism cause an increased formation of metabolites

➔More HAA are released into the medium

n-Propanol in Dependence of Temperature and Pressure



Brauwissenschaft, 208 – 215,1974

Effect of Pressure on Aroma Compounds

		Pressure fermentation	No Pressure
Acetaldehyde	[mg/l]	1,1	0,8
Ethylacetate	[mg/l]	18,0	24,0
N-propanol	[mg/l]	8,0	11,0
Isobutanol	[mg/l]	5	7,0
Isoamyl acetate	[mg/l]	1,1	1,5
Isoamyl alcohol	[mg/l]	31	40,0

MBAA Tech. Quart. 24, 90 – 94, 1987

Formation of Higher Alcohols influenced by FAN

- Free Amino Nitrogen (FAN) may influence content of higher alcohols
- FAN content of wort between 160 and 220 mg/L
- Yeast uptake during fermentation 80 – 120 mg/L
- Remaining in beer 80 – 120 mg/L

Effect of Higher CO_2-Pressure During Fermentation

- Decreased yeast multiplication
- Lower intensity of fermentation
- Lower pH-decrease
- Lower loss of bitter substances
- Lower acetaldehyde formation
- Reduced diacetyl formation
- Reduced higher alcohols formation
- Reduced ester formation
- Less H_2S formation

Effect of Stirring or Intense Convection

- Higher yeast multiplication
- More intensive fermentation
- Faster ph-decrease
- Precipitation of proteins
- Increased losses of bitter substances
- More acetaldehyde formation
- Faster diacetyl formation and reduction
- More higher alcohol formation
- Increased ester formation
- Increased free fatty acid formation
- Decreased head retention

Influence on Fatty Acid Formation

- Yeast strain
- Pitching rate
- Wort composition
- pH-value
- Wort aeration
- Fermentation temperature
- Maturation and storage

Flavours of Fatty Acids

acid/ester	Limit values (mg/l)	Flavour description
Capron acid	7,5	sweaty-, fat-, plantoil like
Capryl acid	7	Fat-, Plantoil-,rancid like
Caprin acid	5	Fat -, wax-, rancid like
Undecan acid	1	-
Laurin acid	0,5	-
Ethyl capronat	0,2	Apple, sweet, fruity-, ester like
Ethyl caprylat	1,1	Apple, sweet, fruity-, ester like

Influences on Acetaldehyde Formation

- Intermediate product of fermentation (“cellar flavour”, “green apple ”)
- In green beer 20 – 40 ppm → during maturation reduction to Ethanol → final concentration in beer 8 – 10 ppm
- Increased formation:
  - Insufficient aeration
  - Intensive fermentation
  - Warm fermentation
  - Pressure fermentation
  - Higher pitching rate

Important Influences on SO_2- Formation

- Wort composition (especially original gravity)
- Oxygen content of pitching wort
- Physiological state of yeast
- Fermentation temperature

Sulphur Compounds

Compound [ppb]	Pils	Flavour threshold
Sulphur dioxide	10,000	20,000
Hydrogen sulphide	5 – 10	5 – 20
Methyl mercaptan	0.1 – 0.5	1.5
Ethyl mercaptan	0.5 – 1.1	2 – 5
Dimethyl sulphide	5 – 150	35 – 70
Diethyl sulphide	0.3 – 10	0.5 – 25
Dimethyl disulphide	0.01 – 0.5	3 – 50
3-Methyl-2-buten-1-thiol	-	0.005

Influences on SO_2 Formation

Increase	Decrease
Increased orig. gravity	High pitching rate
Increased pH	Airation
Lack of Methionin	Addition of lipids

Sulfite is able to mask carbonylgroups ➔ Increase of flavour stability

Stress Factors for Yeast

Fermentation	Crop and pumping	Yeast storage	pitching
Ethanol	Cold shock	Shear forces	Osmotic conditions
Nutrition deficit	Shear forces	Cold shock	Free radicals
Lethal mutations	Anaerobic conditions	Nutrition deficit	
Hydrostatic pressure		Anaerobic conditions	
CO_2			

Ludwig, 2003

Influences of Different Fermentation Factors on Yeast (examples)

- Temperature: yeast is very sensitive against changes by decreasing temperature
  → temperature shock
  temperatur shock by increasing temperatures
  → increased protein synthesis for certain time
- Pressure: → increased partial pressure of CO_2 in beer
  inhibition of certain ways of metabolism and also inhibition of degradation processes
  → possibility of higher fermentation temperatures using higher pressure

Influences of Different Fermentation Factors on Yeast (Examples)

- Extract content: not to high otherwise problems in frementation

→ relation sugar content and aminoacids

- Ethanol: ihibition of yeast growth, fermentation
  decrease of viability and vitality of yeast
- Oxygen: 8-10 mg/l, for yeast growth
  → synthesis of unsaturated fatty acids and lipids

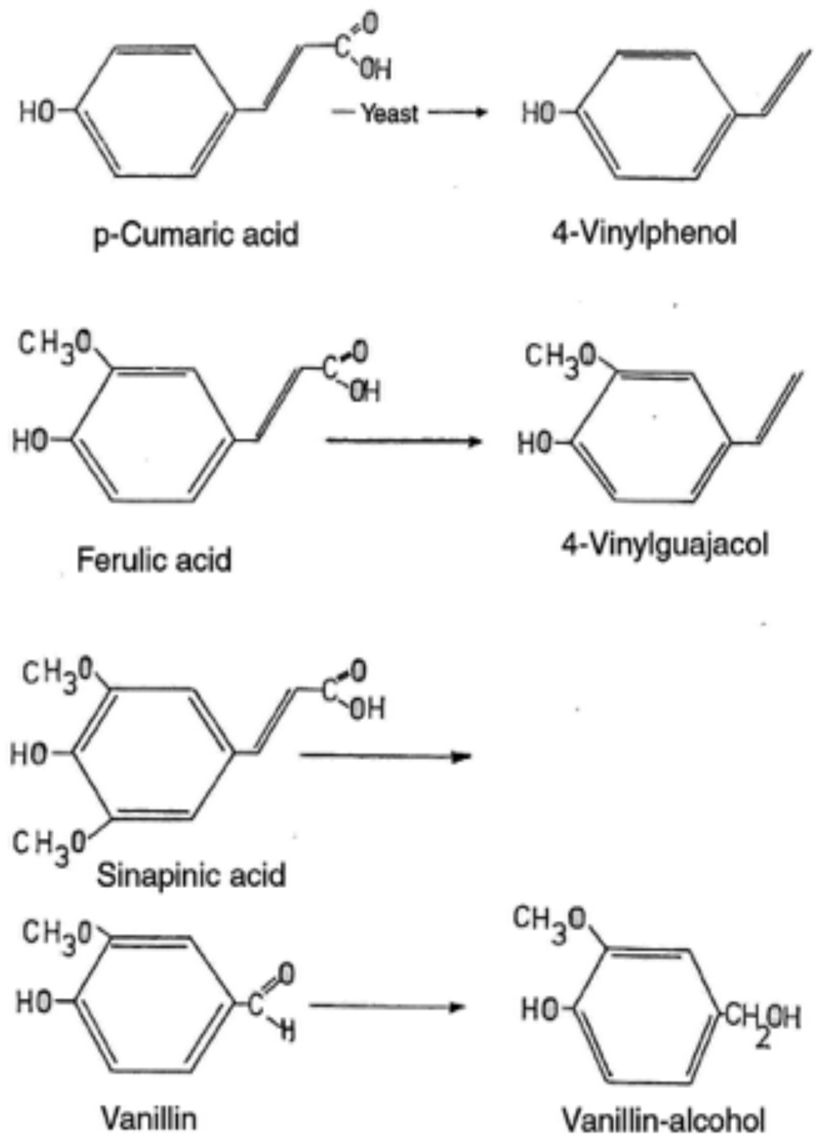
pH - Decrease

- Formation of organic acids
- Uptake of phosphates by yeast ➔decrease of buffer cap.
- Uptake of proteins ➔ decrease of buffer cap.
- CO_2 → no/slight influence
- Acidification of mash increases buffer capacity
- pH decrease ➔ increase of break down of proteins and bittersubstances

Influences on Bitter Substances

Increased elimination	Decreased elimination	No/nearly no influence
Increased aeration	Increased pH	Yeast strain
Intensive main fermentation	Pressure	Pitching rate

Phenolcarbonic Acids & Phenols



Viscosity Decrease in Fermentation

- Gum substances are flocculating caused by the increasing alcohol content
- Alcohol is lower in viscosity
- Sugar content is decreasing

Increased Pitching Rate

- Higher intensity of fermentation
- Increased losses of bitter substances
- Faster diacetyl formation but accelerated reduction
- Increased danger of yeast autolysis
- Lower acetaldehyde formation
- Decreased formation of free short chain fatty acids
- Less content of higher alcohols
- Increased formation of esters
- Decreased yeast multiplication
- Decreased head retention

Increasing Fermentation Temperatures →

- Higher yeast multiplication
- Accelerated fermentation
- Faster pH-decrease
- Higher losses of bitter substances
- Faster diacetyl formation and reduction
- More higher alcohols
- More ester formation
- Increased yeast autolysis
- More free fatty acid formation
- Decreased head retention
- Less high volatile compounds (H_2S, DMS)

Zinc and Fermentation

- In fermentation zinc acts as a coenzyme ➔ essential part of aldolases, dehydrogenases, polymerases and proteases
- Alcoholdehydrogenase (ADH) of yeast consists of four zinc atoms. For the activation of ADH two zinc atoms are needed
- Zinc also provides protection against attack of proteases

Effect of Zinc Deficits

- Reduced activity of enzymes, e.g. ADH
- Insufficient yeast accession
- Higher values of fermentation by products
- Reduced uptake of maltose

- zinc pool size: 6 – 13 mg/100g dry matter
- zinc concentration in wort >0.15mg/l

activity loss of ADH fermenting with a low concentration of zinc in wort

J. Inst Brew. **No. 83**, 17-19, 1977

Yeast's Mineral Supply during Fermentation

Necessary for **fermentation:** → **approx. 0.05 ppm Zn^{2+}**, Necessary for **multiplication:** → **approx. 0.10 ppm Zn^{2+}.**

These amounts are always present in worts from well-modified malt.

Dosage in case of lack of zinc: → **approx. 0.2 mg Zn^{2+}/l,** leading to a total content of: → **approx. 0.3 mg Zn^{2+}/l.**

In concentrations higher than: → **approx. 0.5 mg Zn^{2+}/l** zinc could start to **inhibit** yeast activity.